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Editorial

Retinoblastoma A new challenge to the Knudson's Dogma

In 1971, Alfred Knudson[1] was the first to propose the hypothesis predicting that two mutations (two hits) of key genes in the control of cell division occurring in a retinal neuro-ectodermal cell were necessary for the development of retinoblastoma. According to this hypothesis, the first mutation is a germ line mutation present in all the cells of the body especially in all retinal neuro-ectodermal embryonic cells, while the second mutation is somatic, acquired during fetal life or the first months of neonatal life. In 1973, Comings[2] reinforced this hypothesis by postulating that the two mutations necessary for the development of retinoblastoma correspond to the inactivation of two alleles of the same gene. In 1984, Murphree and Benedict[3] suggested that a single locus existed for all forms of retinoblastoma located in the 13q14 region after observing retinoblastoma cases presenting with dysmorphic features and mental retardation. This observation resulted in genetic studies focusing on this region. In 1986, Friend and associates[4] were the first to identify the retinoblastoma gene and called it Rb1. This was the first representative of a class of cancer genes that restricts the uncontrolled growth of embryonic cells rather than producing cell growth.

The Rb1 gene is composed of 27 exons ranging in size from 31 to 1889 base pairs, the gene product is a 110-kDa nuclear phosphoprotein of 928 amino acids that is normally present in all cells and called RB1. The retinoblastoma gene product is the retinoblastoma protein pRb, which is part of the retinoblastoma family of proteins also called pocket proteins that also include proteins p107 and p130.[5] These proteins share sequence homology in a bipartite domain known as a pocket domain, which mediates interaction with transcription factors from the E2F family during the G1/S transition of the cell cycle. The pRB binds to E2F factors and suppresses their activity consequently blocking progression to the S phase. Inversely, phosphorylation of the pRB releases E2F factors allowing completion of the cell cycle. The pRb protein is involved in regulation of the cell cycle, controlling the termination of cellular differentiation and in exiting of the cell from the cell cycle during development. It also appears to interact with more than 100 different proteins. When pRb is absent cells proliferate uncontrollably, leading to cancer. The interaction between pRB and E2f is regulated by other factors, including cyclin D1, cdk4 and p16. One normal copy of the gene is adequate to prevent tumor formation. Malignant transformation of a retinal cell occurs after both homologous copies of the retinoblastoma gene in that the cell undergoes loss-of-function mutations, supporting Knudson's ''two-hit'' hypothesis.

During the last two decades, advanced techniques in molecular biology provided a better understanding of the oncogenesis and the spectrum of mutations of the RBI gene, allowing identification of up to 932 (and counting) different mutations.[6] The most frequent mutations are nonsense mutations and also missense, splicing mutations, inframe deletions and mutations in the regulatory sequence at the promotor. Hence, investigators can identify relevant phenotype-genotype relationships and provide working hypothesis for mechanisms linking certain mutations to ethnicity, delayed onset of the disease and low penetrance with variable expressivity. Investigations into retinoblastoma oncogenesis have the common denominator that mutations in two alleles or two hits to the RB1 gene are required to develop retinoblastoma based on the Knudson's hypothesis. Until recently, this has been the prevailing Dogma.

The Knudson two-hit Dogma is being challenged by a team lead by Gallie[7] in a large international collaborative study of 1068 cases of unilateral sporadic retinoblastomas involving five laboratories across five countries (Canada, Netherlands, Germany, France, and New Zealand). This group[7] identified 29 cases without Rb mutations (2.9%) and 27 of those have elevated MYCN copy numbers (16 cases with very high copy numbers (28–121)). MYCN is a member of the *MYC* family of transcription factors. Amplification of *MYCN* (*MYCN*A) is most notably associated with neuroblastoma, where it is associated with how aggressive the disease is and resistance to therapy.

These 27 cases labeled MYCN RB were characterized by distinctive histological features. These features include, undifferentiated cells with large nucleoli and indications of necrosis and apoptosis, they have markers of embryonic retina (meeting the definition of retinoblastoma as a blast cell–cell tumor arising from the retina) but they resemble neuroblastoma cells and lack the nuclear molding and Flexner Wintersteiner rosettes seen in classic retinoblastoma. These tumors were large and aggressive and presented at a very early age of diagnosis (median age at diagnosis for MYCN RB was 4.5 months).

The very early presentation of MYCN RB, the absence of RB1 mutations, a functional RB1 protein and high MYCN amplification in a genome with a fairly stable copy number suggest that these tumors arise by somatic MYCN oncogene amplification in a retinal progenitor cell. The method by which MYCN amplification is initiated and whether MYCN amplification alone is sufficient to initiate retinoblastoma, has not been elucidated. In retinal cells, the MYCN protein can support cell division without activation of the E2F family

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of transcription factors. Presumably, unregulated MYCN expression associated with high-level gene amplification in MYCN RB tumors promotes cell division by indirect inactivation of the RB1 protein.

Hence, there is now a new hypothesis of 'one-hit' with amplification of the MYCN oncogene as being sufficient to cause retinoblastoma. Therefore the condition is no more likely to repeat in siblings or cause another cancer in the patients than in the general population. Classic RB, in contrast, often causes other cancers later in life such as Osteosarcoma. Therefore the diagnosis of MYCN RB can save patients from invasive follow-up which includes several examinations under anesthesia. It will also affect the clinical management in cases of MYCN RB since as this study suggests, the disease is particularly aggressive and since the other eye will not become involved, attempt to salvage the eye on the assumption of heritable disease in young children and could incur high treatment morbidity and failure to cure these aggressive oncogene-driven MYCN RB. Therefore, enucleation or surgical removal of the MYCN-RB tumors would be the correct therapeutic course. However, the question remains whether this type of MYCN RB would benefit from an anti-MYCN treatment? This is yet another challenge for the future.

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